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(54) Title: USE OF SUPERCRITICAL FLUIDS TO OBTAIN POROUS SPONGES OF BIODEGRADABLE POLYMERS

(57) Abstract

A method of preparing a biodegradable porous matrix is disclosed, which comprises contacting a biodegradable polymer with a supercritical fluid in a chamber and subsequently reducing the pressure in the chamber in a sharp step. The matrix thus produced has a high porosity and may be used in a variety of pharmaceutical applications such as for surgical implantation or in controlled-release drug delivery systems.

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USE OF SUPERCRITICAL FLUIDS TO OBTAIN POROUS SPONGES OF BIODEGRADABLE POLYMERS

The present invention relates to the preparation of porous materials made of biodegradable polymers.

Polymeric matrices which are both porous and biodegradable are useful in a variety of pharmaceutical applications, such as controlled release drug delivery and surgical implantation. For example, a bone graft substitute comprising a biodegradable porous polymer is described in GB-A-2215209, whilst a controlled drug delivery system comprising porous biodegradable polymeric microspheres is discussed by Sato et al in Pharm.Research, Vol.5, No.1, pp 21-30, 1988. A subcutaneously implanted porous polyvinyl alcohol sponge used to treat rats with basic fibroblast growth factor (bFGF) is reported by McGee et al in J.Surgical Research 45, 145-153 (1988), which treatment is observed to accelerate wound healing in the rats.

A convenient new method of making a porous biodegradable polymer has now unexpectedly been found. Accordingly, the present invention provides a method of preparing a biodegradable porous polymer matrix which comprises contacting a biodegradable polymer with a supercritical fluid in a chamber and subsequently reducing the pressure in the chamber to a value below the critical pressure of the fluid in a sharp step.

By supercritical fluid is meant a gas or liquid above its critical point. The critical point of a substance is the point on a state diagram of temperature plotted against pressure at which there ceases to be a dividing line between the gaseous and liquid states. At the critical point physical properties of the liquid and gaseous states, in particular the densities, are identical. The temperature and pressure values at the critical point may be termed the critical conditions and are constant for a given fluid. The critical conditions for a variety of fluids are listed in Table 1 which follows.

TABLE 1

Critical Conditions for Various Gases and Liquids

	Critical Temperature	Critical Pressure	
	°C	kPa	(atm)
Carbon Dioxide	31.1	7376	(72.8)
Ethane	32.3	4884	(48.2)
Ethylene	9.3	5036	(49.7)
Propane	96.7	4246	(41.9)
Propylene	91.9	4620	(45.6)
Cyclohexane	280.3	4073	(40.2)
Isopropanol	235.2	4762	(47.0)
Benzene	289.0	4894	(48.3)
Toluene	318.6	4114	(40.6)
p-Xylene	343.1	3516	(34.7)
Chlorotrifluoromethane	28.9	3921	(38.7)
Trichlorofluoromethane	198.1	4408	(43.5)
Ammonia	132.5	11280	(111.3)
Water	374.2	22050	(217.6)

A supercritical region may be defined in the state diagram of a substance, as illustrated in Figure 1 of the accompanying drawings. A fluid is in a supercritical condition when subjected to temperature and pressure values falling within this region.

Any supercritical fluid, including any of those listed above in Table 1, may be used in the process of the present invention. Carbon dioxide, at a pressure of at least 7376 kPa and a temperature of at least 31.1°C, is a preferred example.

The biodegradable polymer is suitably brought into contact with the supercritical fluid in the chamber of any standard supercritical extractor or any suitable device, examples being the extractor supplied by Muller Extract Company GmbH, the Sample Preparation Accessory supplied by Milton Roy, or the apparatus shown in Figure 2. In operation, the biodegradable polymer is loaded in the extraction chamber of the supercritical extractor and the supercritical fluid is then applied for a time sufficient to allow supercritical fluid to penetrate the mass of the polymer by diffusion, and possibly dissolve the mass. The time needed will vary according to the fluid and the particular supercritical conditions used, and also according to the amount of material to be penetrated or dissolved. The greater the mass of polymer, the longer will be the time needed for the process to be completed,

assuming all other parameters controlling the process to remain constant. For a mass of polymer of from 1 to 50g, the time is typically of the order of from 3 minutes to 2 hours. For example, when carbon dioxide is the supercritical fluid it is suitably applied to this mass of material for 45 minutes under conditions of 32°C and 7600 kPa (75 atm), or for 1 hour at 32°C and 15200 kPa (150 atm).

Once the process is complete, the pressure inside the supercritical chamber is reduced in a sharp step. This means that the pressure in the chamber is brought to a value below the critical pressure of the fluid, typically in a period of from 0 to 3 minutes. Preferably the period is from 5 to 30 seconds. The pressure may be reduced in this sharp step to any given value below the critical pressure of the fluid; generally it is between the critical pressure and ambient pressure. If this given value is higher or lower than ambient pressure, the pressure in the chamber is restored to ambient subsequently. In one embodiment of the invention the pressure in the chamber is reduced to ambient pressure in the sharp step. By ambient pressure is meant a pressure of about 101.325 kPa (1 atmosphere).

The sharp pressure reduction contributes towards achieving the desired porosity in the material obtained. The greater the difference between the operating pressure

of the supercritical chamber and ambient pressure, the higher is the porosity of the resulting product. The operating temperature of the supercritical chamber can also be adjusted to modify the product porosity; the higher the supercritical temperature employed, the higher is the porosity achieved.

During the process of the invention the operating temperature in the supercritical chamber is controlled. Any suitable control means may be used, for example a water-recirculating bath such as is employed in the Muller extractor, or an air stream such as is employed in the Sample Preparatory Accessory (SPA) apparatus supplied by Milton Roy. The particular operating (supercritical) temperature selected in a given situation will depend partly upon the solubility of the biodegradable polymer in the supercritical fluid since, at a given pressure, this solubility will vary with temperature. It will also partly depend upon the diffusion coefficient of the supercritical fluid in the polymer since, at a given pressure, this coefficient will vary with temperature. As previously indicated, the particular operating temperature employed affects the physical state of the polymer which is achieved.

It is sometimes desirable to operate at the lowest temperature compatible with the solubility of the polymer in the supercritical fluid, and/or with permeation of the

supercritical fluid into the polymer under given supercritical pressure conditions. This may be, for example, when a thermally unstable active ingredient is contained in the polymer. At other times, however, this consideration does not apply and a higher operating temperature may be selected.

Any biodegradable polymer may be used as starting material in the process of the invention, suitable examples including polylactides. The term polylactide designates the general class of polymers which can be prepared from one or more of the following monomers: 3-propiolactone tetramethylglycolide, b-butyrolactone, 4-butyrolactone, pivalolactone, and intermolecular cyclic esters of α-hydroxy butyric acid, α-hydroxyisobutyric acid, α-hydroxyvaleric acid, α-hydroxyisovaleric acid, α-hydroxycaproic acid, α-hydroxy-α-ethylbutyric acid, α-hydroxyisopcaproic acid, α-hydroxy-3-methylvaleric acid, α-hydroxyheptanoic acid, α-hydroxyoctanoic acid, α-hydroxydecanoic acid, α-hydroxymyristic acid, α-hydroxystearic acid, and α-hydroxylignoceric acid. It is most preferred to use lactic acid as sole monomer or lactic acid as the principal monomer with glycolic acid as the comonomer. The latter are termed poly(lactide-co-glycolide) copolymers.

Particularly suitable are polymers prepared from lactic acid alone, glycolic acid alone, or lactic acid and

glycolic acid wherein the glycolic acid is present as a comonomer in a molar ratio of 100:0 to 40:60. It is most preferred to use a poly(lactide-co-glycolide) copolymer having a molar ratio between about 80:20 and 50:50.

Poly(lactide) homopolymers, poly(glycolide) homopolymers and poly(lactide-co-glycolide) copolymers may range in size from 1,000 to 200,000 in molecular weight stated as an average ($\overline{MW}$). The molecular weight of a particular copolymer is independent of its monomeric makeup. For example, a 50:50 copolymer can have a molecular weight which falls anywhere within this range. Therefore polymers can be varied both as to their monomer composition as well as their molecular weight.

Examples of other suitable biodegradable polymers include: polyanhydrides, for example as described in US-A-4,757,128, Biomaterials 1983, Vol. 4, pages 131-133, J. Biomedical Materials Research, Vol. 19, pages 941-955 (1985), J. Biomedical Materials Research, Vol. 20, pages 51-64 (1986) or Biomaterials 1986, Vol. 7, pages 364-371; and polyacetals, polyketals and poly(orthoesters), for example as described in Polym.Sci.Technol. 1986, Vol. 34, Polymer Med. 2, pages 357-365.

The biodegradable polymeric starting material may be in the form of microspheres, pellets, a matrix of any geometrical shape, or in any other form. When microspheres

or any other of the aforementioned starting materials are used these may be, if desired, pre-loaded with an active ingredient so that the process of the invention yields, in one step, a porous spongy material carrying an active ingredient. Microspheres of a poly(lactide-co-glycolide) copolymer or of a polylactide homopolymer are particularly suitable.

When the biodegradable polymer is in the form of microspheres, these are prepared in a conventional manner. A standard method involves dispersing the biodegradable polymer in an organic solvent, a suitable example being dichloromethane, and adding a silicone oil dropwise to the dispersion with stirring. The microspheres thus obtained are then washed and hardened by treatment with a non-polar hydrocarbon solvent such as pentane or heptane.

The active ingredient contained in the microspheres may be any drug used in surgery, therapy or prophylaxis. Examples include: peptides and proteins, in particular immunomodulators such as Thymic Humoral Factor; growth factors such as basic Fibroblastic Growth Factor, acid Fibroblastic Growth Factor, Epidermal Growth Factor, Human Growth Factor, Insulin Like Growth Factor, Platelet Derived Growth Factor, Nerve Growth Factor and Transforming Growth Factor; antitumorals such as BCNU or 1,3-bis(2-chloro-ethyl)-1-nitrosourea, daunorubicin, doxorubicin, epirubicin, idarubicin, 4-demethoxydaunorubicin

3'-desamine-3'-(3-cyano-4-morpholinyl) - doxorubicin, 4-demethoxydaunorubicin-3'-desamine-3'-(2-methoxy-4-morpholinyl)-doxorubicin, etoposide and teniposide; hormones such as LHRH and LHRH analogues; and steroideals for birth control and/or antitumoral action such as medroxyprogesterone acetate or megestrol acetate.

If forms of biodegradable polymer other than microspheres are used, such as pellets or matrices of any geometric shape, these may also, if desired, be pre-loaded with an active ingredient such as any of those listed above.

The porous biodegradable polymeric material obtained in accordance with the invention may be used as a surgical implant, for example to aid the reconstitution of tissues after surgical removal of a tumour or after trauma injuries. Such an implant has immediate utility in filling anti-aesthetic voids caused by the surgery or injury, giving support for newly-growing tissue which is able to fill the pores of the material. The biodegradable material itself, as it disintegrates in vivo, will then gradually be replaced by the new tissue. The time required for the degradation of the biodegradable material is related to the composition of the polymer(s), the molecular weight of the polymer, the relative proportions of comonomers (in the case of a copolymer(s)), and the physical state. The process of reconstitution of the damaged tissue is enhanced

if the porous polymer is loaded with a suitable growth factor such as any of those listed earlier, chosen according to the particular application.

The porous biodegradable material is also particularly useful in the treatment of tumours which are not removable. In this case the material is loaded with an anti-tumour agent, such as any of those listed earlier, and implanted at the site of the tumour. An example is the treatment of the glioblastoma multiform in the brain.

The porosity (P) of the material produced in accordance with the invention may be calculated, in percentage terms, using the following equation:

$$P = [1-(D1/D2)] \times 100$$

in which D1 is the apparent density of the material and D2 is the true density, measured by the standard method of helium picnometry. A porosity of up to as high as 74% is achieved in accordance with the invention.

Besides giving rise to a highly porous product the process of the invention has the important advantages that, since it allows a non-oxidising agent to be used at a relatively low temperature (e.g. CO_2 above 31.1°C), it is relatively non-hazardous and non-toxic and does not cause degradation of any active ingredient contained in the biodegradable polymer starting material. In addition, it is easy to carry out on a large scale and is therefore of economic importance.

The following Examples further illustrate the invention.

Reference Example 1: Preparation of biodegradable microspheres

2g of poly (D,L) lactide, of molecular weight 100,000 and intrinsic viscosity of 1 (Boehringer Ingelheim) were dissolved in 80 ml of CH_2Cl_2. 0.8 ml of phosphate buffer (pH 7.4, I=0.1) were dispersed finely in the CH_2Cl_2 phase with an Ultra-Turrax stirring turbine equipment, operating at 4000 rpm with a dispersing tool (type G45F). 60ml of silicone oil Dow Corning Fluid 200 were added dropwise under continued stirring.

The microspheres obtained were washed and hardened with 2l of n-heptane, then desiccated under vacuum at 35°C for 24 hours, and sieved. The microspheres obtained had a mean diameter of about 80 μm.

The n-heptane residual content was about 17%.

Reference Example 2

The process of Reference Example 1 was carried out using poly ((D,L) lactide-co-glycolide) copolymer, having a lactide: glycolide ratio of 75:25, a molecular weight of 18000 and intrinsic viscosity of 0.8 (Boehringer Ingelheim) instead of the poly (D,L) lactide.

The n-heptane residual content was about 10%.

Reference Example 3

The process of Example 2 was repeated using n-pentane in place of n-heptane.

The n-pentane residual content was about 5%

Reference Example 4: Preparation of biodegradable microspheres loaded with an active ingredient

2g of poly (D,L) lactide of molecular weight 100,000 and intrinsic viscosity of 1 (Boehringer Ingelheim) were dissolved in 80ml of CH_2Cl_2. 200mcg of basic fibroblast growth factor was dissolved in 0.8ml of phosphate buffer (pH 7.4, I=0.1) and the aqueous phase dispersed finely in the CH_2Cl_2 with an Ultra-Turrax stirring turbine apparatus, operating at 4000 rpm with a dispersing tool (type G45F). 60ml of silicone oil Dow Corning Fluid 200 were added dropwise under continous stirring. The microspheres obtained were washed and hardened with 2l of n-heptane, then desiccated under vacuum at 35°C for 24 hours, and sieved.

The microspheres obtained had a mean diameter of about 80 μm. The n-heptane residual content was about 17%.

EXAMPLE 1: Preparation of porous biodegradable material

1.5 g of the microspheres obtained by the process of Reference Example 1 were loaded in the extraction chamber of a supercritical extractor (Muller Extract Company GmbH).

CO_2 was applied at 15200 kPa (150 bar) and 35°C for 1 hour.

The CO_2 was taken from a bomb (cylinder) and loaded into the extractor. The pressure of 15200 kPa (150 bar) was applied by means of a mechanical pump. The temperature was kept at 35°C during the process by means of a recirculating water bath. After 1 hour the pressure was reduced to ambient pressure in 10 seconds. The extraction chamber was opened and a spongy material was obtained.

The porosity of the material was determined by the equation:

$$P = [1-(D1/D2)] \times 100$$

where D1 is the apparent density and D2 is the true density measured by helium picnometry and found to be about 76%.

The residual content of n-heptane was 100 ppm.

EXAMPLE 2

The process of Example 1 was repeated but using the following CO_2 supercritical conditions: 7600 kPa (75 bar), 32°C, 45 min.

The material obtained was found to have a porosity of about 64%.

EXAMPLE 3

The process of each of Examples 1 and 2 was repeated, but using the biodegradable microspheres loaded with active ingredient prepared in Reference Example 4. A spongy bio-

degradable materials loaded with the active ingredient was obtained, in each case, having a porosity analogous to that achieved with the unloaded material obtained in Examples 1 and 2.

Reference Example 5

The process of Example 1 was repeated, but using the following CO_2 subcritical conditions: 5066 kPa (50 bar), 32°C, 15 min. The material obtained was characterized with a porosity of about 54%.

CLAIMS

1. A method of preparing a biodegradable porous matrix which comprises contacting a biodegradable polymer with a supercritical fluid in a chamber and subsequently reducing the pressure in the chamber to a value below the critical pressure of the fluid in a sharp step.

2. A method according to claim 1 wherein the biodegradable polymer is a polylactide homopolymer or a poly(lactide-co-glycolide) copolymer.

3. A method according to claim 1 or 2 wherein the biodegradable polymer is in the form of microspheres.

4. A method according to any one of the preceding claims wherein the biodegradable polymer is loaded with an active substance.

5. A method according to any one of the preceding claims wherein the supercritical fluid is carbon dioxide at a temperature of at least 31.1°C and a pressure of at least 72.8 atmospheres.

6. A surgical implant comprising a biodegradable porous matrix prepared by the method as claimed in claim 1.

7. A controlled release drug delivery system comprising a biodegradable porous matrix prepared by the method as claimed in claim 1 and loaded with an active ingredient.

8. A method of preparing a biodegradable porous matrix substantially as hereinbefore described in any one of Examples 1 to 4.

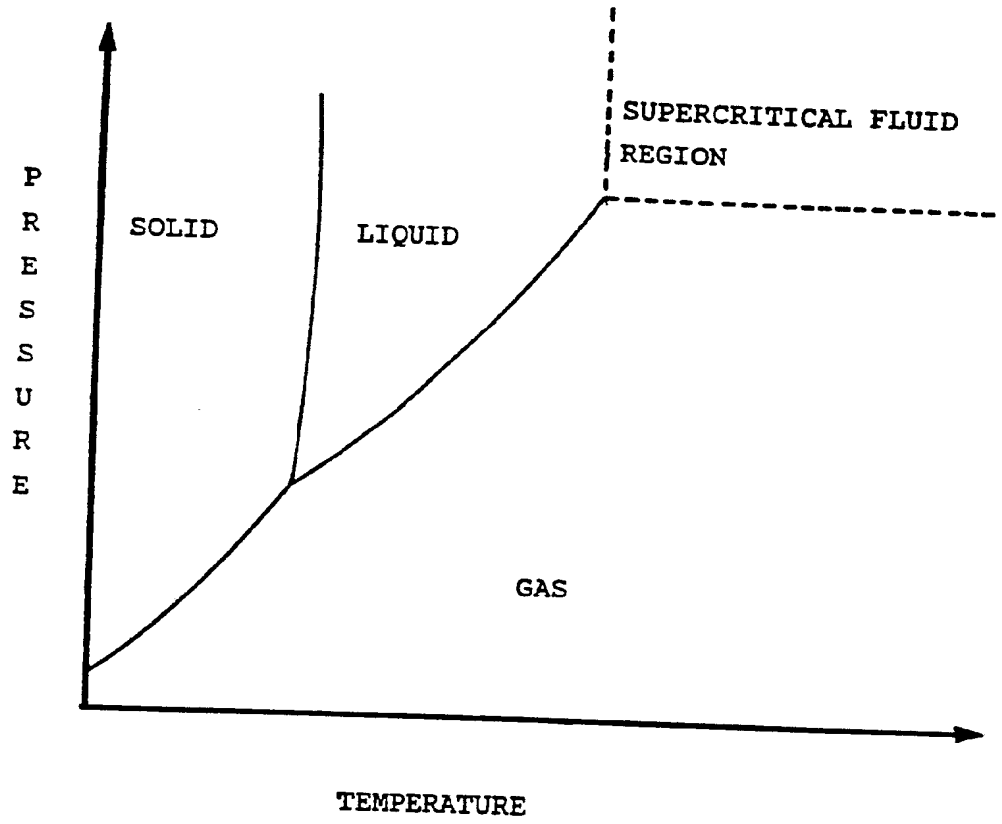
SUPERCRITICAL FLUID
REGION
PRESSURE
SOLID
LIQUID
GAS
TEMPERATURE

FIGURE 1

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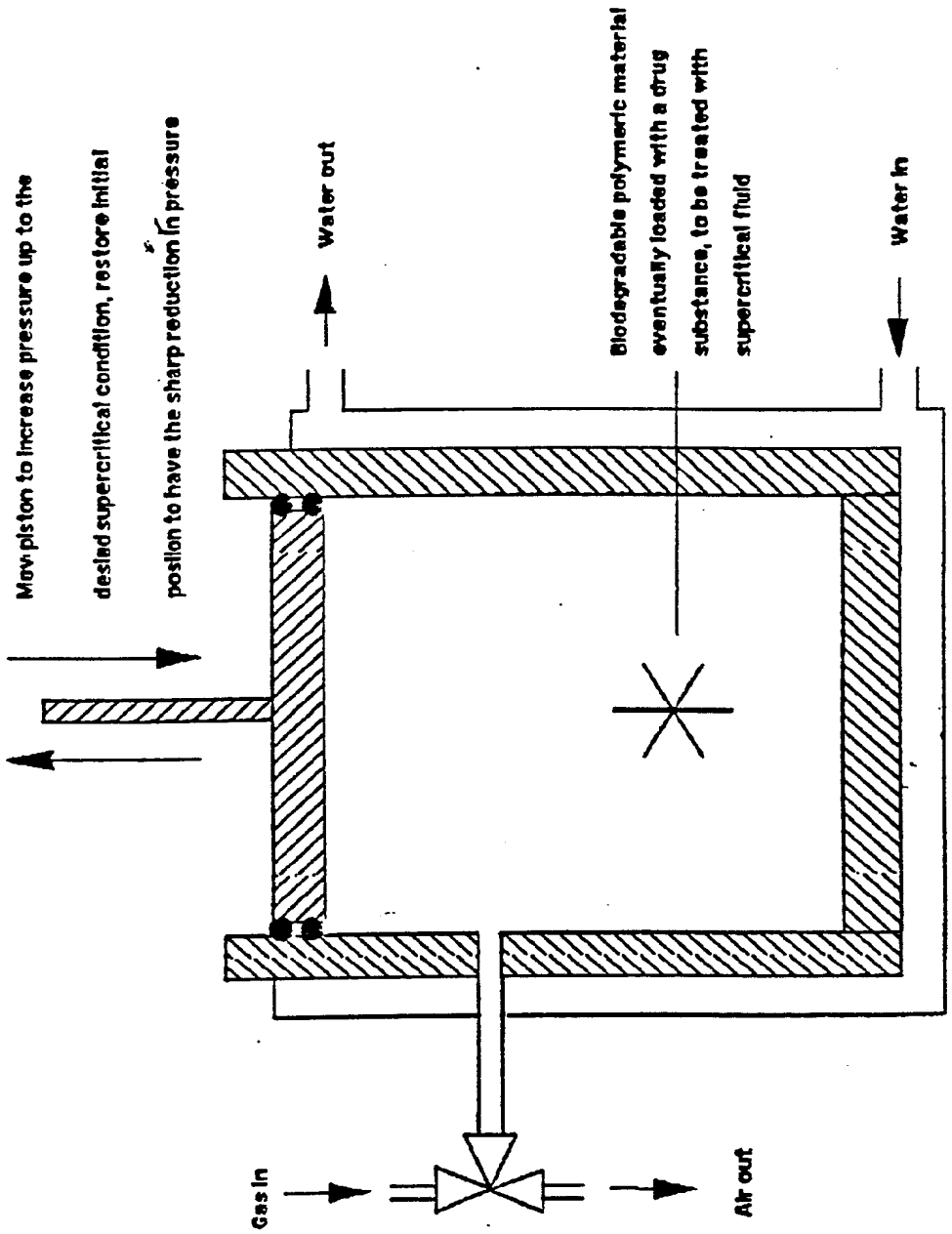
Move piston to increase pressure up to the
desired supercritical condition, restore initial
position to have the sharp reduction in pressure
Water out
Biodegradable polymeric material
eventually loaded with a drug
substance, to be treated with
supercritical fluid
Water in
Gas in
Air out

FIGURE 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 90/01895

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C08J9/18 ; A41K9/16 ; //C08L67:04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C08J ; A41K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	EP,A,113903 (JAPAN STYRENE PAPER CORPORATION) see page 2, line 29 - page 2, line 37; claims 1-5, 9, 10	1-8
Y	US,A,4719246 (DU PONT DE NEMOURS) see column 4, line 65 - column 5, line 3; claims 1-16	1-8
A	EP,A,251631 (ANDERSON) see claims 1-10	1-8
A	EP,A,204476 (INTERNATIONAL MINERALS AND CHEMICALS CORP.) see claims 1-15	1-8
A	US,A,3681270 (DYNAMIT NOBEL A.G.) see claims 1, 2, 6	1-8

° Special categories of cited documents : [10]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
31 JANUARY 1991	18 FEB 1991
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	OUDOT R.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 90/01895

SA 41814

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US-A- 4766182	23-08-88
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		AU-B- 590565	09-11-89
		AU-A- 5767286	27-11-86
		JP-A- 61277629	08-12-86
US-A-3681270	01-08-72	None	

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